International Journal of Environment, Agriculture and Biotechnology
Vol-7, Issue-4; Jul-Aug, 2022

Journal Home Page Available: https://ijeab.com/
Journal DOI: 10.22161/ijeab

Peer Reviewed

Priming Applications with Mosquito (Diptera: Culicidae) Larva Rearing Water in White Cabbage Seeds (*Brassica oleracea* L. var. *capitata* F. *alba* cv. Yalova 1)

Nihan Şahin[1*], Levent Arın[2], Mehmet Uludağ[3], Zafer Şakacı[4], Sırrı Kar[5]

[1,2,3]Department of Horticulture, Faculty of Agriculture, Tekirdağ Namık Kemal University, Tekirdağ, Turkey
[4,5]Department of Biology, Tekirdağ Namık Kemal University, Tekirdağ, Turkey

Received: 22 Jul 2022; Received in revised form: 12 Aug 2022; Accepted: 16 Aug 2022; Available online: 20 Aug 2022

Abstract— *The starting material with good quality is a portent of a satisfactory and good harvest in vegetables grown from seedlings such as cabbage, a good beginning makes a good ending. Seed pre-applications promote uniform and rapid germination/emergence, leading to strong seedling formation. In this study, bio-based larval rearing water was used as an organic priming agent and its effect was compared with the effects of hydro priming. As a result of the study, the effects of both priming applications on germination and emergence were superior to the untreated control in terms of mean time and vigor, but there were no differences according to germination and emergence percentages. According to mean time of germination organic primed seeds germinated in the shortest time with 2.45 days compared to hydro primed seeds (2.77 days) and control (3.48 days). Likewise, this, organic primed seeds were emergence in 6.80 days, hydro primed seeds were emergence in 7.19 days and control seeds were emergence in 7.39 days. Vigor index of germination was 19.09 in organic priming, but it was 17.48 in hydro priming and 14.56 in control. On the other hand, vigor index of emergence was 6.39 in organic priming, 5.67 in control and, 5.28 in hydro priming applications. In addition, organic priming came to the fore among organic priming and hydro priming. It is thought that this positive effect may be due to the nitrogen and organic carbon content of larval rearing water.*

Keywords— ***Cabbage, Hydro priming, Larval rearing water, Organic priming.***

I. INTRODUCTION

Main purpose of priming applications is stimulating the biochemical activities via increasing water uptake of seeds for uniform and faster germination and emergence [1]. For these purposes, water, some salts, or various chemicals can be use. In addition to these, the use of bio-based/organic materials in priming applications have been investigated in recent years[2-5]. In this studies, organic priming of seeds defined as the safe and practical approach and which organics are safe, ecofriendly, economical, and easily available. Also, these applications provide especially hardiness to various unfavorable environment conditions such as extreme temperature and moisture. It induces faster germination in seeds and higher seedling vigor leading to higher crop productivity. These effects presented with various research. For example; in cluster bean the main benefits of organic seed treatments include increased phosphate levels, nitrogen fixation and root development [6]. In *Capsicum* species organic priming applications promote larger seedlings with greater fresh and dry weight for all species and suggested that priming may be used to enhance pepper seedling performance [7]. In watermelon organic priming provided faster and earlier seedling development [8].

Vigor is a major seed trait, which designates the potential of uniform emergence and seedling development even under undesirable growing conditions. Uniform seedling establishment is mandatory in all vegetable crops and can be achieved by improving seed quality [9]. There is some approach to enhance of the seed quality before sowing,

even if seed quality improved by breeding, for various reasons such as unfavorable storage conditions, seed age, insufficient labor etc. In addition of these priming application provide a recovery chance for aged or damaged seeds [2]. There are different seed vigor improve techniques, priming is one of those. Heterogeneous germination and seedling emergence have direct influence on field performance of the plants either of the transplanted vegetable crops[9].

Cabbage is widely growth nearly all countries, mostly Asian and Northern Europe. It can be use as fresh or cooked, as pickled, as medicine or feed [10]. Cabbage contains plenty of various minerals, water and fiber, as well as is very rich in vitamins K and C [11]. In 2020, cabbages and other brassicas was produced 71 million tonnes in the world at 2.4 million ha. Turkey is the 11th cabbage producer in the producer countries according to production quantity (852.000 tonnes).

Seedlings are widely used in cabbage culture. Irregular seed quality leads to extended emergence time and non-uniformed seedlings of cabbage. That variable sized seedlings, when transplanted, produce variable sized cabbage heads which cannot be harvested and marketed at one time [9]. Thornton and Powell [12] reported improvements in seed quality of Brassica oleracea via hydro priming. Hydro priming, imbibition of seeds before sowing, is an easy and inexpensive method to improve seed emergence and seedling uniformity [13].

Gravit female of *Culex pipiens* Linnaeus, 1758 (Diptera: Culicidae) (northern house mosquito) lays eggs in a raft in a boat-shaped structure, consisting of several hundred eggs. As the breeding site, this species prefers natural or artificial containers that accommodate up to several liters of water. The embryonic development takes about 2–7 days or more depending on the temperature, and immediately after, the larval emergence occurs. The aquatic process, consisting of four larval and one pupal stages, is usually completed within one week or more, depending on temperature and other environmental drivers, such as food level (organic debris, bacteria, protozoans, fungi and other microorganisms) and the density of larvae in the water [14]. To date, a limited number of studies have been conducted to determine the effects of mosquito larvae on the rearing water, particularly touching on the subject from a microbiological point of view. Depending on the species and environmental conditions, these studies revealed that presence of mosquito larvae can alter the microorganismal composition of water and can enhance, reduce, or have little effect on the total bacterial abundance [15-18]. In most of the relevant studies, a few mosquito species have been focused, such as *Aedes triseriatus* [15, 17], *Culex nigripalpus* [19], and *Culex restuans* [18]. The effects of larvae on the rearing water have been mostly explained through the trophic cascade [18, 19]. However, it has also been emphasized that mosquito larvae can play an important role in the structuring of microbial food sources, and release of some nitrogenous wastes by the larvae may be one of the crucial effects in this phenomenon [18]. As far as we know, there is no detailed data on the effects of *C. pipiens* larvae on the rearing water, the specific molecules that are possibly excreted/secreted by mosquito larvae or microorganisms exposed to the larvae, and the potential effects of these possible molecules on any animals or plants.

Objectives of the study reported here were (1) the evaluation of bio-based rearing water of mosquito species *C. pipiens* effects on germination and emergence of cabbage seeds and (2) to compare its' effect with hydro priming.

II. MATERIAL AND METHODS

In this study, commercial standard variety "Yalova 1" white head cabbage seeds treated with Thiram used as plant material. With take into considerations of several previous investigations [13, 20, 21], seeds were subjected to two different priming solutions [hydro priming (PW) and larval rearing water (LRW)] and three different soaking durations [one day soaking (1DS), three days soaking (3DS) and soaking until 1% of seeds radicle appearing (approximately 1.5 days, 1G)] and in addition of those non-pre-applied seeds used as control (C). After these applications, all seeds were dried to initial seed weight except C groups seeds. All seeds threated with Mancozeb 64% (Syngenta, Ridomil Gold MZ 68WG) to protection against fungal contaminations before sowing. Experimental design was randomized complete plots with three replications.

To obtain the rearing water of *C. pipiens* larvae, seven containers made of hard plastic and each containing two liters of water were used. In each container, 200 first instar larvae were placed and fed with fry food, considering the relevant instructions and warnings [14]. The emerged adult mosquitoes were collected daily. Eventually, an average of 197.2 (range 194-200) adult mosquitoes, 103.1 (range: 96-110) male and 94.1 (range: 90-104) female, emerged from each container. All the larval rearing water from the seven containers were mixed in a stock container, and all the trials were carried out using this mix.

The larval rearing water evaluated for its some features. According to the total organic carbon analysis, it was included 18 mgL^{-1} total organic carbon (38 mgL^{-1} total carbon) and 25 mgL^{-1} total nitrogen. According to the

physicochemical measurements it has 0.65 mS cm^{-1} EC and 8.40 pH values.

Plant Growth Chamber (ALC 800) was used for the conduct of the study settled at 24±1° C, 65±5% RH and, darkness conditions. Germination and emergence parameters were evaluated in the study. For germination tests 50 seeds were planted into each petri dishes (9 cm Ø, plexiglass) and, for emergence tests 50 seeds were sown at a depth approximately 0.5 cm in growing medium filled trays (8x25x50 cm, PE) per replicate. The commercial peat recommended to produce vegetable seedlings was used as a growing medium (Klassmann Potground-H, Doktor Tarsa Inc., Antalya, Turkey). It had pH of 6.0, EC-value of 0.40 dS m^{-1}, Added amount of fertilizer (NPK fertilizer 14:10:18): 1.5 kg m^{-3}.

The counts were done daily, and it was accepted as germinated when approximately 2 mm radicle appeared and as emergenced when the cotyledons were parallel to medium surface. Observations conducted until there was not germination and emergence in three consecutive days.

Percentage of germination (GP) and emergence (EP) was calculated by proportion and arcsine square root transformation values used for statistical analyses, but real values of germination/emergence percentages presented in Tables. Mean germination time (MT_G) and mean emergence time (MT_E) were calculated according to Formula 1. Vigor indexes of germination (VI_G) and emergence (VI_E) were calculated according to Mereddy, Wu [22] (Formula 2).

$$MT = \frac{\sum n.t}{\sum n} \qquad \text{(Formula 1)}$$

n = number of newly emergenced seedling/germinated seed at a time t, t = days from sowing/planting, and Σ n = total emergenced seedling/germinated seed.

$$VI = \left(\frac{G1}{D1}\right) + \left(\frac{G2}{D2}\right) + \cdots + \left(\frac{GL}{DL}\right) \qquad \text{(Formula 2)}$$

G1 = number of emerged/germinated seeds (first count), D1 = number of days to first count, GL = number of emerged/germinated seeds (last count), and DL = number of days to last count.

All data were subjected to analysis of variance (ANOVA) and mean value were compared with the LSD test. Statistical analyses are conducted in R statistical analysis software version 4.1.0. [23] and Agricolae library [24].

III. RESULTS AND DISCUSSION

According to ANOVA results in GP among durations, in MT_G application main effect, in VI_G both among durations and application main effect (Table 1).

In MT_E application main effect, and in VI_E application main effect were significant but EP was non-significant (NS) statistically. Differences among all the combinations (applications x durations) were statistically significant (Table 2).

Percentage of germination ranged between 60.00% and 88.00% and there were not significantly differences among combinations except PW 3DS. While combinations were statistically different there was no difference in application main effect (Table 1).

According to mean time of germination, applications main effect was statistically significant. LRW applications' seeds germinated in the shortest time with 2.45 days compared to PW (2.77 days) and control (3.48 days) applications. Combinations of applications and conditions were significantly different. When combinations of LRW and 1DS, 3DS, 1G and combination of PW and 1DS placed same group (a), PW and 3DS, 1G combinations placed same group (ab) and C placed group by itself (b).

Vigor index of germination (VI_G) is significantly different according to the combination of applications also application main effect. As GP and MT_G, combinations of LRW and application main effect of LRW showed best results compared to PW and Control applications (Table 1). Distribution of germination by days showed at Fig.1. According to the distribution of germination graph all of seeds of LRW 1DS, 3DS and 1G conditions nearly germinated at first seven days.

Table 1. Germination percentage (GP), mean time of germination (MT_G) and vigor index of germination (VI_G) seeds of primed with water (PW) and larval rearing water (LRW) one day (1DS), three days (3DS) and until the appearance 1% of seeds radicle (1G).

Applications	Conditions	GP (%)	MT_G	VI_G
PW	**1DS**	**86.67a**	**2.47a**	**20.53a**
	3DS	**60.00b**	**2.93ab**	**12.83b**
	1G	**83.33a**	**2.93ab**	**19.09a**
	Mean	**76.67**	**2.77AB**	**17.48AB**

LRW	**1DS**	**78.00a**		**2.41a**		**17.80ab**	
	3DS	**80.00a**		**2.54a**		**19.53a**	
	1G	**81.33a**		**2.42a**		**19.95a**	
	Mean		**79.78**		**2.45A**		**19.09A**
	Control	**88.00a**	**88.00**	**3.48b**	**3.48B**	**14.56ab**	**14.56B**
	LSD value	**8.344 (α:0.05)**		**0.556 (α:0.01)**	**0.777 (α:0.001)**	**5.330 (α:0.001)**	**3.595 (α:0.1)**

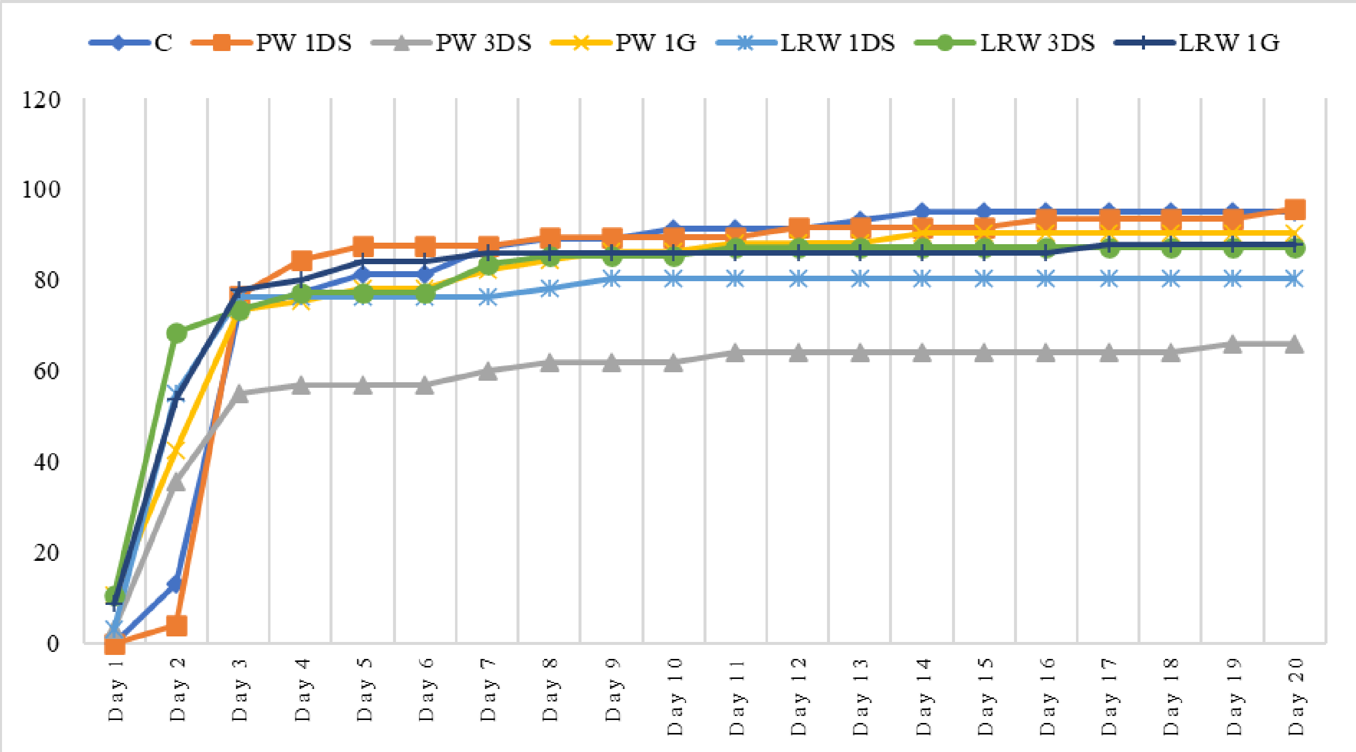

Fig.1. Distribution percentages of germination by days

Emergence parameters were showed a similar pattern to germination parameters. In all parameters combinations of LRW showed the best results. According to the EP LRW (1DS, 3DS and 1G) and C grouped together (a). One percent of seeds germinate in LRW showed the highest emergence rate (85.33%) and three days soaking of PW showed the lowest emergence rate (56.67%). Application main effect not statistically significant in EP. Main time of emergence was statistically different for conditions and application main effects. One day and three days soak of LRW showed the shortest mean time (6.69, 6.78 days) and control was showed the longest mean time (7.39 days).

According to application main effect of mean time of emergence PW (7.19 days) and LRW (6.80 days) were superior to control application (7.39 days). Vigor index of emergence showed comparable results with VI_G. Application main effect of VI_E was the most favorable in LRW when it compared to PW and Control (

Table 2). Distribution of emergence by days showed at Fig.2. The sixth day was the busiest day of the emergence trial, most of seeds were emergence at sixth day for all conditions.

Table 2. Emergence percentage (EP), mean time of emergence (MT_E) and vigor index of emergence (VI_E) seeds of primed with water (PW) and larval rearing water (LRW) one day (1DS), three days (3DS) and until the appearance 1% of seeds radicle (1G).

Applications	Conditions	EP (%)		MT_E (days)		VI_E	
PW	**1DS**	**82.67a**		**6.87ab**		**6.28a**	
	3DS	**56.67b**		**7.36cd**		**4.12b**	
	1G	**74.00a**		**7.32bcd**		**5.44a**	
	Mean		**71.11**		**7.19A**		**5.28B**
LRW	**1DS**	**84.67a**		**6.69a**		**6.69a**	
	3DS	**78.00a**		**6.78a**		**6.03a**	
	1G	**85.33a**		**6.93abc**		**6.46a**	
	Mean		**82.67**		**6.80A**		**6.39A**
	Control	**79.33a**	**79.33**	**7.39d**	**7.39B**	**5.67a**	**5.67AB**
	LSD value	**9.188 (α:0.05)**		**0.394 (α: 0.1)**	**0.488 (α:0.05)**	**1.085 (α:0.05)**	**1.119 (α:0.1)**

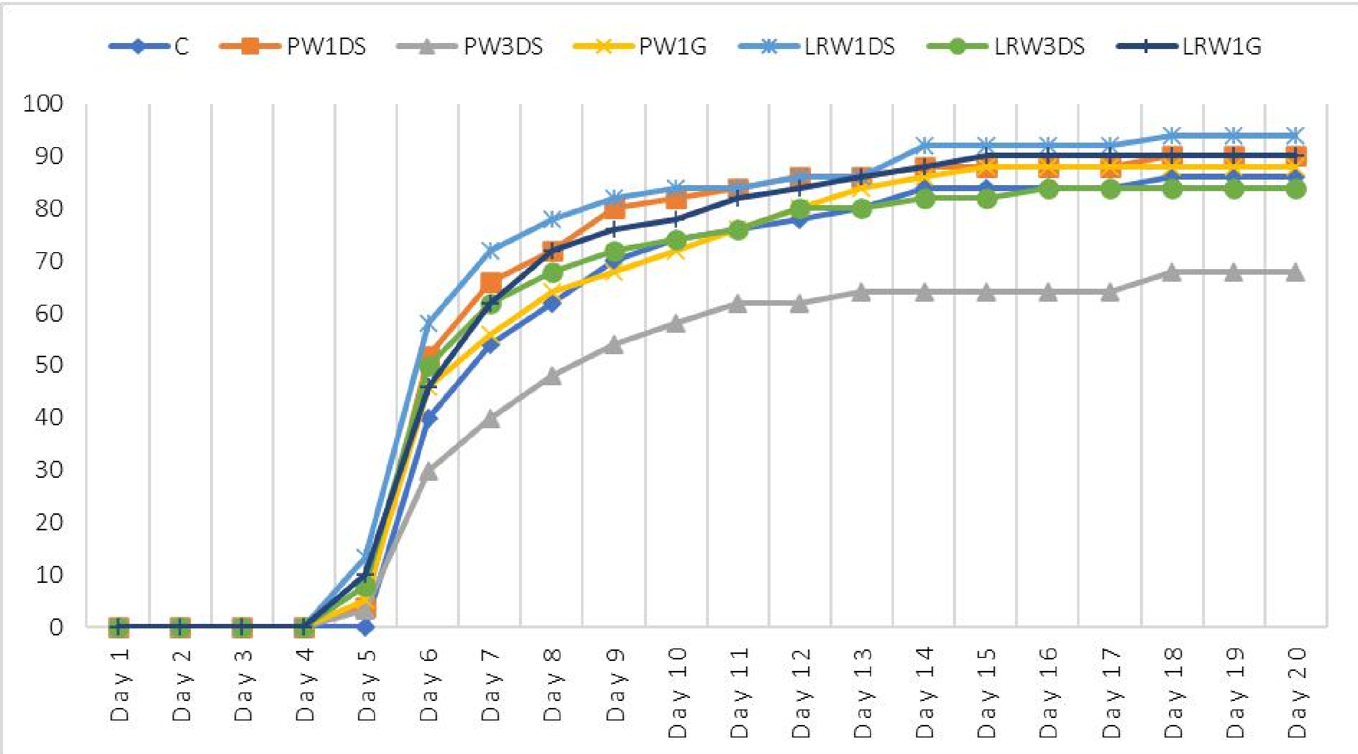

Fig.2. Distribution percentage of emergence by days

Among all combinations, PW 3DS has the lowest germination and emergence performance. The reason of this situation could be long priming time. In the seeds of 1G application, radicles were appeared in approximately 1.5 days. In PW 3DS application, seeds subjected to hydro priming longer time than other applications. The reason of

that it could be seeds in PW 3DS application insufficient oxygen. On the other hand, it was not same effect seen in the LRW 3DS. It could be thought that the reason why it was due to the positive effect of LRW on the germination and emergence performances (Figure 1 and 2).

Özkaynak, Yüksel [8] were use laurel, thyme and seaweed extracts and PEG_{6000} for comparing chemical and organic priming applications in watermelon seeds and showed that extracts efficient as PEG_{6000} in germination rates. Özkaynak, Orhan [25] were also use laurel, thyme, and seaweed extracts for organic priming in tomato and pepper seeds and suggest that while in pepper seeds seaweed and laurel extracts showed the highest germination rates, in tomato seaweed and thyme showed the best results. In this study, PW and LRW both showed higher performance compared to control, however LWR has the best performance in all parameters.

Takoliya, Patel [26] determined that green leafy vegetables seeds treated with seaweed extract as a bio-priming were showed higher vigor, germination percentage, seedling length and, seed stamina index than untreated seeds. Sivritepe and Sivritepe [21] and Sivritepe, Şentürk [20] were use seaweed extract in pepper seeds for organic priming applications and suggest that the seaweed extract applied seeds showed superior vigor compared to control group. In our study LRW has been same effect as bio-priming agent.

It were used different concentrations of cow urine in cluster bean for organic priming applications by Ambika and Balakrishnan [6]. Researchers suggest that the cow urine (2%) can be recommended as organic seed priming for increasing the vigor in cluster bean. It can be thought that the LRW used in our study showed similar effects depending on the organic carbon and nitrogen content.

IV. CONCLUSION

Seed priming is a very important, efficient, applicable and inexpensive method to increase the germination, emergence, the growth, as well as the productive capability of vegetable crops. There is no single method applicable to all species to initiate germination metabolism without radical protrusion. For the identification of species-specific methods water, inorganic salts, sugars, solid medium with water and nutrients, beneficial microbes, micronutrients, hormones, rhizobacteria, and organic sources are used as priming agents for seeds [2].

In this study both hydro priming and larval rearing water as organic primig used for improve seed germination and emergence properties in cabbage seeds. Both methods positively induced the mean time of germination/emergence and, the vigor index of germination compared to control group. However, there was no difference among the applications according to germination and emergence percentages. Comparing LRW and PW applications, it was seen that LRW gave superior results in MT_G, VI_G and VI_E.

Primed seeds can show in high germination and seedling emergence rates, vigorous early growth, early flowering, maturity and higher yields than unprimed seeds [26]. The results of the present study showed that seed priming with LRW improve the germination and emergence of cabbage seeds. Hence, priming with LRW could yield to uniform seedling production. In future studies, examining the performance of seedlings obtained from LRW-treated seeds will help to better understand the effects of this material.

REFERENCES

[1] Alan, Ö. and H. İlbi, *Ekim öncesi tohum uygulamaları (priming) yapılan tohumlarda depolama.* TÜRKTOB Türkiye Tohumcular Birliği Dergisi, 2018. **28**: p. 20-22.

[2] Sher, A., et al., *Methods of seed priming*, in *Priming and pretreatment of seeds and seedlings*. 2019, Springer. p. 1-10.

[3] Satani, H., S. Verma, and H. Solanki, *A Review on Effects of Pre-sowing Organic Priming on Seed Germination.* Research and Reviews: Journal of Environmental Sciences, 2021. **3**(2): p. 1-8.

[4] Waqas, M., et al., *Advances in the concept and methods of seed priming*, in *Priming and pretreatment of seeds and seedlings*. 2019, Springer. p. 11-41.

[5] Paparella, S., et al., *Seed priming: state of the art and new perspectives.* Plant cell reports, 2015. **34**(8): p. 1281-1293.

[6] Ambika, S. and K. Balakrishnan, *Enhancing germination and seedling vigour in cluster bean by organic priming.* Scientific Research and Essays, 2015. **10**(8): p. 298-301.

[7] Mavi, K., *Evaluation of organic priming to improve the emergence performance of domesticated Capsicum species.* Seed Science and Technology, 2018. **46**(1): p. 131-137.

[8] Özkaynak, E., et al., *Karpuzda (Citrullus lanatus (Thunb.) Matsum. & Nakai) organik priming uygulamaları.* Erciyes Üniversitesi Fen Bilimleri Enstitüsü Fen Bilimleri Dergisi, 2015. **31**(2): p. 149-155.

[9] Batool, A., K. Ziaf, and M. Amjad, *Effect of halo-priming on germination and vigor index of cabbage (Brassica oleracea var. capitata).* Journal of environmental and Agricultural Sciences, 2015. **2**(7): p. 1-8.

[10] Gruszecki, R., et al., *Cabbage in Polish folk and veterinary medicine.* South African Journal of Botany, 2022. **149**: p. 435-445.

[11] Haque, K., et al., *Yield and nutritional quality of cabbage as affected by nitrogen and phosphorus fertilization.* Bangladesh journal of scientific and industrial research, 2006. **41**(1): p. 41-46.

[12] Thornton, J. and A. Powell, *Short-term aerated hydration for the improvement of seed quality in Brassica oleracea L.* Seed Science Research, 1992. **2**(1): p. 41-49.

[13] Mal, D., et al., *Seed priming in vegetable crops: A review.* Int. J. Curr. Microbiol. App. Sci, 2019. **8**(06): p. 868-874.

[14] Becker, N., et al., *Subfamily Anophelinae*, in *Mosquitoes*. 2020, Springer. p. 169-192.

[15] Walker, E.D., et al., *Nutrient dynamics, bacterial populations, and mosquito productivity in tree hole ecosystems and microcosms.* Ecology, 1991. **72**(5): p. 1529-1546.

[16] Paradise, C.J. and W.A. Dunson, *Effects of sodium concentration on Aedes triseriatus (Diptera: Culicidae) and microorganisms in treeholes.* Journal of medical entomology, 1998. **35**(5): p. 839-844.

[17] Kaufman, M.G., et al., *Effects of larval mosquitoes (Aedes triseriatus) and stemflow on microbial community dynamics in container habitats.* Applied and environmental microbiology, 1999. **65**(6): p. 2661-2673.

[18] Muturi, E.J., C. Dunlap, and C.E. Caceres, *Microbial communities of container aquatic habitats shift in response to Culex restuans larvae.* FEMS microbiology ecology, 2020. **96**(7): p. fiaa112.

[19] Duguma, D., M.G. Kaufman, and A.B. Simas Domingos, *Aquatic microfauna alter larval food resources and affect development and biomass of West Nile and Saint Louis encephalitis vector Culex nigripalpus (Diptera: Culicidae).* Ecology and Evolution, 2017. **7**(10): p. 3507-3519.

[20] Sivritepe, H.Ö., B. Şentürk, and S. Teoman, *Biber tohumlarında yapılan organik priming ve kurutma uygulamaları fide kalitesi ve performansını iyileştirmektedir.* Uludağ Üniversitesi Ziraat Fakültesi Dergisi, 2015. **29**(2).

[21] Sivritepe, N. and H.Ö. Sivritepe, *Organic priming with seaweed extract (Ascophyllum nodosum) affects viability of pepper seeds.* Asian Journal of Chemistry, 2008. **20**(7): p. 5689.

[22] Mereddy, R., et al., *Solid Matrix Priming Improves Seedling Vigor of Okra Seeds.* Proceedings of the Oklahoma Academy of Science, 2000. **80**: p. 33-37.

[23] Team, R.C., *R: A language and environment for statistical computing.* 2013.

[24] De Mendiburu, F. and M.F. de Mendiburu, *Package 'agricolae'.* R Package, version, 2019. **1**(3).

[25] Özkaynak, E., et al., *Biber ve domates tohumlarında organik priming uygulamaları.* Black Sea Journal of Agriculture, 2020. **3**(4): p. 301-307.

[26] Takoliya, H.H., R.V. Patel, and N. Brahmbhatt, *Improving Green Leafy Vegetables Seed Germination Using Bio-Priming Treatment.* International Journal of Recent Scientific Research, 2018. **9**: p. 24774-24778.